DATA EVALUATION RECORD

NNI-0001 (FLUBENDIAMIDE)

Study Type: OPPTS 870.6300 [§83-6], Developmental Neurotoxicity Study in Rats

Work Assignment No. 4-1-124 L; formerly 3-1-124 L (MRID 46817228)

Prepared for

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DATA EVALUATION RECORD

STUDY TYPE: Developmental Neurotoxicity Study – Rat; OPPTS 870.6300 ('83-6); OECD 426 (draft)

PC CODE: 027602 **DP BARCODE:** D 331553 (SB)
TXR# 0054319

TEST MATERIAL (PURITY): NNI-0001 (Flubendiamide; 97.3% a.i.)

SYNONYMS: N^2-[1,1-dimethyl-2-(methylsulfonyl)ethyl]-3-iodo-N^1-[2-methyl-4-[1,2,2,2-tetrafluoro-1-(trifluoromethyl)ethyl]phenyl]-1,2-benzenedicarboxamide

CITATION: Sheets, L.P., R.G. Gilmore, and H.E. Hoss (2006) A developmental neurotoxicity screening study with technical grade NNI-0001 in Wistar rats. Bayer CropScience LP, Stilwell, KS. Laboratory Study No.: 04-D72-VK, February 17, 2006. MRID 46817228. Unpublished.

SPONSOR: Bayer CropScience LP, 2 T.W. Alexander Dr, Research Triangle Park, NC

EXECUTIVE SUMMARY - In a developmental neurotoxicity study 2006 (MRID 46817228) technical grade NNI-0001 (Flubendiamide; 97.3% a.i., Lot/Batch # 1FH0019M) was administered to approximately 30 mated female Wistar rats per dose in the diet at nominal dose levels of 0, 120, 1200, or 12,000 ppm from gestation day (GD) 6 through lactation day (LD) 21. Doses were adjusted during lactation to achieve a more consistent dosage throughout exposure. The mean daily intake during gestation and lactation was 0, 9.9, 99.5, and 979.6 mg/kg/day. Dams were allowed to deliver naturally and were killed on LD 21. On postnatal day (PND) 4, litters were standardized to 8 pups/litter. Subsequently, 1 pup/litter/group (at least 10 pups/sex/dose when available) were allocated to subsets for functional observational battery (FOB), motor activity, acoustic startle response, learning and memory evaluation, and neuropathological examination.

Maternal Effects:
All dams survived to scheduled sacrifice. No treatment-related clinical signs were observed during gestation or lactation. No adverse effects were observed in any FOB parameter at any time point.

During gestation, slight increases ($p \leq 0.05$) were noted in body weight (↑4-5%) and body weight gains (GD 0-20, ↑11%) at 12000 ppm. During lactation, body weights were increased ($p \leq 0.05$) by 4% at 120 and 1200 ppm on LD 0 and by 4-6% at 1200 and above on LD 21. Body weight gains (LD 0-21, calculated by reviewers) were also

increased by 17-18% at 1200 ppm and above. Food consumption in the treated groups was similar to controls during gestation and lactation. These differences in body weight and body weight gain were not considered to be treatment-related as the body weights of the treated animals were slightly higher than controls at the beginning of the study and minor increases in weight are not considered to be adverse. In the LD21 dams, increases ($p \leq 0.05$) in absolute (↑26-34%) and relative (to body, ↑20-28%) liver weight were observed at 1200 ppm and above.

Reproductive parameters were similar to controls in all dose groups.

Pup effects.:
Offspring pre-weaning body weights were decreased ($p \leq 0.05$) at 12,000 ppm (↓9% both sexes) at PND 21. Body weight gains were decreased ($p \leq 0.05$) during several pre-weaning intervals at 12,000 ppm (↓12-20%, males and ↓11-20%, females). Overall combined pup body weight gains (calculated by reviewers) were decreased by 11% at 12,000 ppm.

Sexual maturation – The day of preputial separation was delayed ($p \leq 0.01$) in the 1200 and 12,000 ppm males (47.5 and 48.7 treated vs. 44.9 controls). The day of vaginal patency was delayed ($p \leq 0.01$) in the 12,000 ppm females (35.3 treated vs. 32.6 controls). Only 2 pups in the 12,000 ppm group did not display pupil constriction on PND 21.

Functional observational battery – Treatment-related FOB effects were limited to ocular lesions (corneal opacity, dark red eyes, and/or enlarged eyes) at 12,000 ppm (1-2 males and 1-4 females). The lesions were observed beginning on PND 21 and persisted until PND 60 in both sexes. All other FOB findings were considered incidental and unrelated to treatment.

At 12,000 ppm, treatment-related ocular lesions were noted in pups of both sexes as follows. During pre-weaning (PND 0-21), the following effects on the eyes (# of litters affected/20-29 vs. 0 controls) were observed: (i) enlarged eyeball (1-9 litters on PND 15-21); (ii) corneal opacity (2-3 litters on PND 16-21); (iii) dark red (1-6 litters on PND 15-21); and (iv) exophthalmia (1 litter on PND 20-21). Throughout post-weaning (PND 22-72), the following effects on the eyes (# of animals affected/65-66 vs. 0 controls) were noted: (i) enlarged (9 males/12 females); (ii) general opacity (8 males/10 females); (iii) red (4 males/8 females; and (iv) exophthalmia (2 males). Additionally in the 1200 ppm males, one pup displayed enlarged eye, general opacity, and exophthalmia. No compound-related effects were noted at 1200 ppm in the females or at 120 ppm in either sex.

Microscopic examination – At 12,000 ppm, the following compound-related microscopic effects (# affected/10 vs. 0 controls, unless otherwise stated) were noted in the eye and optic nerve: (i) retinal degeneration (2 males and 1 female); (ii) hemorrhage (3 males vs. 1 control); (iii) cataract (2 males and 1 female); and (iv) atrophy of the optic nerve (3 males and 1 female). No other treatment-related microscopic lesions were noted at any dose in either sex.

Maternal LOAEL = 99.5 mg/kg/day based on increased liver weights. Increased liver weight in isolation is not considered an "adverse" effect, but considering the consistent observation of liver toxicity (e.g., centrilobular hepatocyte fatty change, hypertrophy,

increase in liver enzymes, foci of cellular alterations) demonstrated across multiple durations and species at similar doses, the weight-of-evidence supports this effect as an "adverse" finding and thus, a firm basis for the LOAEL. Maternal NOAEL= 9.9 mg/kg/day.

Offspring LOAEL = 99.5 mg/kg/day based on delayed balanopreputial separations. The Offspring NOAEL = 9.9 mg/kg/day.

This study is classified (**acceptable/non-guideline**) and satisfies the guideline requirement; OPPTS 870.6300, '83-6, OECD 426 (draft) for a developmental neurotoxicity study in rats.

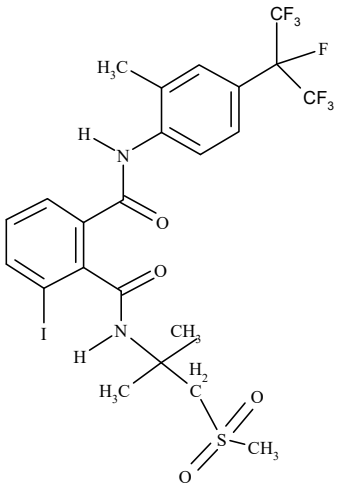
COMPLIANCE - Signed and dated Data Confidentiality, GLP Compliance, Flagging, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: NNI-0001

Description: White powder
Lot/Batch #: 1FH0019M
Purity: 97.3% a.i.
Stability: The test material was shown to be stable in the diet for up to 7 days at room temperature or 28 days frozen.
CAS # of TGAI: 272451-65-7
Structure:



2. Vehicle – Diet

3. Test animals (P)

Species:	Rat	
Strain:	Wistar HAN CRL:WI (GLX/BRL/HAN) IGSBR	
Age at study initiation:	Approximately 12 weeks at cohabitation	
Mean group weights on GD 0:	230.2-237.9 g females only	
Source:	Charles River Laboratories, Inc. (Raleigh, NC)	
Housing:	During gestation and lactation, individual dams and litters were kept together in plastic cages with corn cob bedding. The week following weaning, the remaining F_1 pups were kept individually in stainless steel wire-mesh cages.	
Diet:	Rodent Lab Chow #5002 (PMI Nutrition International, St. Louis MO), *ad libitum*, except during neurobehavioral testing	
Water:	Tap water, *ad libitum*, except during neurobehavioral testing	
Environmental conditions	**Temperature:**	18-26°C
	Humidity:	30-70%
	Air changes:	10/hr
	Photoperiod:	12 hrs dark/ 12 hrs light
Acclimation period:	At least 6 days	

B. PROCEDURES AND STUDY DESIGN

1. In-life dates - Dams received the test material starting on 6/20/04 and ending on approximately 7/25/04.

2. **Study schedule -** The maternal animals were mated and assigned to study. The test substance was administered to the dams from gestation day (GD) 6 through lactation day (LD) 21. Pups were weaned on postnatal day (PND) 21, after which time all animals received untreated diet. On PND 4, the litters were randomly standardized to 8 pups/litter (with equal sexes where possible) to reduce the variability. All litters not selected for further observations and all P females without a litter were sacrificed, and were discarded without further examinations. F_1 pups remained on study until PND 75 (±5 days, study termination).

3. **Mating procedure -** Females were paired 1:1 with males of the same strain and source for a maximum of four consecutive days. Each female was examined daily during the mating period to identify sperm cells in a vaginal smear or the presence of a copulatory plug. The day that sperm or a plug was found was designated gestation day (GD) 0, and each female was housed individually in a plastic nesting box.

4. **Animal assignment -** Time-mated females were randomly assigned to test groups as shown in Table 1. Offspring were assigned to testing subgroups at the time of litter standardization on PND 4. Dams were assigned to functional observation testing as shown.

TABLE 1. Study design [a]

Experimental Parameter	Subset	Dose (ppm)			
		0	120	1200	12,000
Maternal Animals					
No. of dams assigned	NA	30	30	30	30
Mean daily intake (mg/kg/day)	NA	0	9.9	99.5	979.6
FOB (GD 13 and 20)	NA	30	30	30	30
FOB (LD 11 and 21)	NA	10	9	10	10
Offspring (F_1) [b]					
Motor activity (PND 13, 17, 21, 60±2)	A	1 pup/litter (~16/sex)	1 pup/litter (~16/sex)	1 pup/litter (~16/sex)	1 pup/litter (~16/sex)
Acoustic startle habituation (PND 22, 60±2)	B	1 pup/litter (~16/sex)	1 pup/litter (~16/sex)	1 pup/litter (~16/sex)	1 pup/litter (~16/sex)
Passive avoidance (PND 22 and 29)	C	1 pup/litter (~16/sex)	1 pup/litter (~16/sex)	1 pup/litter (~16/sex)	1 pup/litter (~16/sex)
Water maze (PND 60±2 and 7 days later)	C	1 pup/litter (~16/sex)	1 pup/litter (~16/sex)	1 pup/litter (~16/sex)	1 pup/litter (~16/sex)
FOB (PND 4, 11, 21, 35±1, 45±1, 60±2)	C	1 pup/litter (~16/sex)	1 pup/litter (~16/sex)	1 pup/litter (~16/sex)	1 pup/litter (~16/sex)
Perfusion, neuropathology, and morphometric analysis (PND 21)	D	10/sex	10/sex	10/sex	10/sex
Brain weight (PND 75±5)	A, B, C	10/sex	10/sex	10/sex	10/sex
Ophthalmologic examination (PND 50-60)	A, B, C	~10/sex	~10/sex	~10/sex	~10/sex
Perfusion and neuropathology (PND 75±5)	A, B, C	same animals selected for ophthalmologic examination	same animals selected for ophthalmologic examination	same animals selected for ophthalmologic examination	same animals selected for ophthalmologic examination

a Data obtained from pages 19, 20, and 41 of the study report.
b Unless otherwise indicated, 1 male or female pup/litter was used (~16 [minimum of 10]/sex/dose, representing at least 20 litters).
NA Not applicable

5. **Dose selection rationale -** Dose levels were chosen based on the results of a two-generation reproduction study in Wistar rats (MRID 46817216; reviewed concurrently), in which NNI-0001 was administered in the diet at nominal concentrations of 20, 50, 2000, or 20,000 ppm. At 2000 ppm and above, treatment-related effects noted in the parental generation females included increased liver and thyroid weights as well as histopathological effects in both organs. Based on these results, doses of 120, 1200, and 12,000 ppm were chosen for the current study.

6. **Dosage preparation, administration, and analysis -** Formulations were prepared weekly by mixing appropriate amounts of test substance with the diet. The test diets were stored frozen (-20°C) until use. Dietary formulations were provided to the dams for *ad libitum* consumption weekly throughout the exposure period (GD 6 through LD 21). Dietary concentrations were adjusted (reduced) during lactation, relative to gestation, to maintain a more constant level of exposure (mg/kg/day) throughout the treatment period. F_1 animals were not directly supplied with the test diets. Prior to initiation of the study, stability for up to 7 days at room temperature or for up to 28 days frozen and homogeneity (top, middle, bottom) at concentrations of 40 and 12,000 ppm (which bracketed those in the current study) were determined. Actual concentration at each dose was tested for each batch of dietary formulations used in the current study.

Results

Homogeneity analysis (% relative standard deviation): 1.98-5.56%

Stability analysis (range as % of Day 0)
At room temperature for 7 days: 93-100%
Frozen for 28 days: 94-99%

Concentration analysis (% of nominal):

Dose (ppm)	% Nominal
120	108
1200	106
12,000	108

The analytical data indicated that the mixing procedure was adequate and that the variation between nominal and actual dosage to the animals was acceptable.

C. OBSERVATIONS

1. In-life observations

a. Maternal animals – Cage-side checks for mortality, moribundity, and clinical signs of toxicity were conducted at least once daily for maternal animals. Detailed clinical examinations were performed daily during exposure (GD 6 through LD 21).

Animals presumed to be pregnant (approximately 30/dose) were observed on GD 13 and 20 and a minimum of 10 dams/dose were observed on LD 11 and 21 as part of a functional observational battery (FOB). The FOB included, but was not limited to, the following observations (with severity scoring).

	FUNCTIONAL OBSERVATIONS
X	Signs of autonomic function, including: 1) Ranking or degree of lacrimation and salivation 2) Presence or absence of piloerection and exophthalmos, 3) Ranking or count of urination and defecation 4) Pupillary function such as constriction of the pupil in response to light, or a measure of pupil size 5) Degree of palpebral closure
X	Description, incidence, and severity of any convulsions, tremors, or abnormal movements.
X	Description and incidence of posture and gait abnormalities.
X	Description and incidence of any unusual or abnormal behaviors, excessive or repetitive actions, emaciation, dehydration, hypotonia or hypertonia, altered fur appearance, red or crusty deposits around the eyes, nose, or mouth, and any other observations that may facilitate interpretation of the data.

The technicians performing the FOB were 'blind' as to the animal's treatment group. Several technicians were used during the FOB; however, it was stated that evidence of inter-observer reliability (positive control studies) is maintained at the laboratory. No further information concerning the performance of the FOB was provided.

Individual maternal body weight and food consumption data were recorded weekly throughout gestation (GD 6, 13, and 20), on the day of delivery (LD 0), and on LD 4, 7, 14, and 21.

b. Offspring

1. **Litter observations -** The day of completion of parturition was designated as PND 0. Live pups were counted, sexed and weighed individually for each litter on PND 0, 4, 11, 17, and 21. At least once daily, all surviving offspring were examined cage-side for gross signs of toxicity, mortality, or morbidity.

   On PND 4, litters were standardized (using random procedures) to a maximum of 8 pups/litter (4/sex/litter, as nearly as possible). Pups not chosen for the F_1 groups and dams that had insufficient pups were killed and discarded without further examination.

2. **Developmental landmarks -** Beginning on PND 38, male offspring were examined daily for preputial separation. Beginning on PND 29, female offspring were examined daily for vaginal patency. The age of onset was recorded, and the pups were weighed when vaginal patency or preputial separation was first noted. In addition, all pups were tested for the presence of pupil constriction on PND 21.

3. **Post-weaning observations -** After weaning on PND 21, offspring were examined twice daily for mortality and clinical observations were recorded daily. In addition, detailed clinical observations were recorded weekly during post-weaning. Body weights were recorded weekly.

4. **Neurobehavioral evaluations -** Observations and the schedule for those observations are summarized as follows from the report.

i. **Functional observational battery (FOB) -** On PND 4, 11, 21, 35 (±1 day), 45 (±1 day), and 60 (±2 days), selected pups (approximately 16/sex/dose; Subset C) were observed outside the home cage according to procedures outlined for the dams. The neonates were not evaluated in the open field on PND 4 and 11.

ii. **Motor activity testing -** Activity was evaluated in approximately 16 pups/sex/dose (Subset A) on PND 13, 17, 21, and 60 (±2 days). Motor and locomotor activity were measured by testing animals in figure eight mazes using a Columbus Instruments Universal Maze Monitoring System (Columbus, OH). Broad-spectrum background noise [74±2dB(A)] was provided, and the light intensity (100±70 Lux) over each maze was verified daily. Each test session was 60 minutes in duration, and consisted of 6 ten-minute intervals. Motor activity was measured as the number of beam interruptions that occurred during the test session. Locomotor activity was measured by eliminating consecutive counts for a given beam. Habituation was evaluated as a decrement in activity over consecutive intervals of the test session.

iii. **Acoustic startle habituation -** Acoustic startle habituation testing was performed on approximately 16 pups/sex/dose (Subset B) on PNDs 22 and 60 (±2 days). A Coulbourn Instruments Integrated Startle Response Test System (Allentown, PA) was used to conduct the test and collect the data. The test session consisted of 50 trials that began following a 5 minute adaptation period at ambient noise levels. The rats were then presented with the startle-eliciting stimulus (50 msec burst [0 msec rise/fall] of broad-spectrum 'white' noise [~118 dB (lin)]) at 10-second intervals. The response amplitude was recorded (maximum value on the curve) and the baseline (animal's body weight) was subtracted. The latency to peak is the time (msec) following the onset of the stimulus when the peak response amplitude occurs.

iv. **Learning and memory testing -** Learning and memory testing was performed on approximately 16 pups/sex/dose (Subset C). Passive avoidance testing was performed on PNDs 22 and 29; water maze testing was performed on PND 60 (±2 days) and again seven days later. For both tests, only animals that demonstrated acquisition on the first day were tested for retention seven days later.

Passive avoidance test - The test was conducted using a Coulbourn Instruments Shuttle Cage System (Allentown, PA). Each shuttle cage consisted of two equal sized compartments separated by a wall that supported a (guillotine-type) door. The walls of one compartment were covered with black film (dark-side), and the other compartment was illuminated with a high-intensity lamp. The floor of the dark-side consisted of a grid of stainless-steel bars. Movement of the animal across the doorway was detected with a photocell system. A

Coulbourn solid state scanning shock generator was used to deliver a brief (0.5 sec) pulse of mild (0.5 mA) shock to the grid floor when the animal crossed into the dark compartment. After adaptation, individual animals were placed into the lighted compartment of a conditioning apparatus facing toward the light. After approximately 60 seconds, the trial began with the light being illuminated to signal the beginning of the trial and the door separating the two compartments opening, so that each rat was provided access to the dark-side of the cage. When the rat crossed into the dark compartment, the door automatically closed, the shock was delivered, and the light was switched off, signaling the end of the trial. At that time the animal was returned to the holding cage to await the next trial. If the rat failed to cross within 180 seconds, it was returned to the holding cage and the latency assigned an arbitrary score of 180. The procedure was repeated until either the rat remained in the lighted compartment for 180 seconds on two consecutive trials or until 15 trials had elapsed, whichever occurred first. Rats that failed to meet the criterion during the learning phase were assigned a value of 15 for the trials-to-criterion variable. The test was repeated one week later. For the second trial, rats were placed in the illuminated side of the apparatus, given a 20 second acclimation period, and the latency to enter the dark side was recorded. Animals that either failed to reach criterion within 15 trials, or failed to cross during the first two trials during acquisition, were excluded from the retention phase of the experiment.

Water maze - A Plexiglas M-maze containing 7.5 inches of water (22±1EC) was used. On each test trial, the rat was placed into the starting position at the base of the M-maze stem, located between the two lateral arms. On the first trial (learning trial), the rat was required to enter both arms of the maze before being provided access to the exit ramp to escape the water and then removed from the maze. The initial arm chosen on this learning trial was designated the incorrect goal during the subsequent 15 trials (maximum). Rats that failed to make a correct goal choice within 60 seconds in any given trial were guided to the correct goal with the exit ramp and then removed from the water. Between trials, the animal was returned to a transport cage to wait for the next trial. The inter-trial interval was approximately 15 (±5) seconds. Each rat was required to reach a criterion of five consecutive errorless trials to terminate the test session. The maximum number of trials in any test session was fifteen. Latency to choose the correct goal or the maximum 60-second interval was recorded for each trial, as was the number of errors during each trial. Animals that satisfied the above criteria within the 15 trial limit were tested for retention seven days following acquisition. Animals that failed to reach criterion during acquisition were excluded from the retention phase of the experiment. The correct goal and criterion were the same in both sessions.

5. **Ophthalmology -** Animals that were selected for perfusion (minimum of 10/sex/dose) were subjected to ophthalmoscopic examinations at approximately 50-60 days of age. The eyes of each animal were examined with a slit lamp microscope and an indirect ophthalmoscope equipped with a condensing lens.

6. **Cholinesterase determination –** Cholinesterase activity was not determined.

7. Postmortem observations

a. Maternal animals - Maternal animals were sacrificed by carbon dioxide asphyxiation on either GD 24 (rats that did not deliver) or LD 21 (following weaning). The liver and thyroid were weighed and the dams were discarded without further necropsy.

b. Offspring - The offspring selected for perfusion on PND 21 (subset D) and at study termination (subsets A-C), as well as those selected for fresh brain weight determinations (approximately 10/sex/group from subsets A-C) were examined grossly.

The animals selected for perfusion on PND 21 (Subset D) and at termination (Subsets A-C) were anaesthetized with pentobarbital (50 mg/kg i.p.), and then perfused with a buffered sodium nitrite flush followed by *in situ* fixation with 1.0% (w/v) glutaraldehyde and 4% (w/v) formaldehyde in phosphate buffer. Only the brain (with olfactory bulbs) was collected from the perfused animals on PND 21. Upon study termination, the brain and spinal cord, eyes (with optic nerves), selected peripheral nerves (sciatic, tibial, and sural), the gasserian ganglion, gastrocnemius muscle, and both forelimbs were collected. All tissues were post-fixed in 10% buffered formalin. The brain from each animal was weighed, sectioned (8 coronal sections), and examined microscopically. The brain, spinal cord, cauda equina, eyes, optic nerves, and gastrocnemius muscle were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. All other collected tissues were embedded in resin, sectioned, and stained with modified Lee's stain. Additionally, the brain sections selected for morphometric measurements were stained with Luxol fast blue/cresyl violet. The following (CHECKED X) tissues, to be examined microscopically, were collected from perfused animals at study termination:

	CENTRAL NERVOUS SYSTEM		PERIPHERAL NERVOUS SYSTEM
	BRAIN	X	**SCIATIC NERVE**
X	Olfactory bulb region		Proximal
X	Olfactory bulb section		Distal
X	Forebrain (optic nerve) section		
X	Forebrain (optic chiasma) section		**OTHER**
X	Midbrain section	X	Sural nerve
X	Mesencephalon	X	Tibial nerve
X	Cerebellum/Pons		Peroneal nerve
X	Medulla oblongata	X	Cervical dorsal root ganglion
	SPINAL CORD	X	Cervical dorsal root fibers
X	Cervical swelling	X	Cervical ventral root fibers
X	Thoracic swelling		Thoracic dorsal root ganglion
X	Lumbar swelling		Thoracic dorsal root fibers
	OTHER		Thoracic ventral root fibers
X	Gasserian ganglion	X	Lumbar dorsal root ganglion
	Pituitary gland	X	Lumbar dorsal root fibers
X	Eyes (with optic nerve)	X	Lumbar ventral root fibers
X	Skeletal muscle (gastrocnemius)		
X	Cauda equina		

Only tissues from the control and 12,000 ppm groups were subjected to microscopic examination and morphometric analysis. Prior to sectioning, the anterior to posterior length of the cerebrum and cerebellum were measured. The following brain sections were measured: 1) frontal cortex thickness; 2) parietal cortex thickness; 3) caudate putamen horizontal width; 4) hippocampal gyrus thickness; and 5) cerebellum height.

Additionally, the entire head was collected from animals culled on PND 4 and preserved in Bouin's fixative. In animals not selected for perfusion (Subset D) that were at least 21 days of age, the eyes were collected and preserved in 10% formalin for possible microscopic evaluation.

D. DATA ANALYSIS

1. **Statistical analyses –** In general, continuous data were initially assessed for equality of variance using Bartlett's test. Group means with equal variances were analyzed further using ANOVA, followed by Dunnett's test as necessary. Group means with unequal variances were analyzed using non-parametric procedures (Kruskal-Wallis ANOVA followed by the Mann-Whitney U test). The level of significance was set at p#0.05, with the exception of Bartlett's test which was set at p#0.001. The following data sets were analyzed by specific statistical procedures:

Parameter	Statistical Procedure
FOB continuous data, motor and locomotor total session activity, acoustic startle response amplitude data (peak amplitude), water maze (latency data)	ANOVA followed by Dunnett's, as necessary
FOB categorical data	General Linear Modeling, Categorical Modeling, Dunnett's test, and Analysis of Contrasts
Interval motor and locomotor activity data	Repeated measures ANOVA (test interval and test occasion) followed by ANOVA and Dunnett's, as necessary
Acoustic startle response amplitude, block data	Repeated measures ANOVA (test block) followed by Dunnett's, as necessary
Passive avoidance (latency data)	Wilcoxon Test for time to failure
Passive avoidance (number of trials-to-criterion) Water maze (number of trials-to-criterion and number of errors)	Kruskal-Wallis and Wilcoxon tests for the acquisition phase and Fisher's Exact Test for retention
Brain weight, gross brain measurements	ANOVA or Kruskal-Wallis
Microscopic brain measurements	ANOVA and/or 2-tailed T-test
Micropathology	Chi-Square and One-tailed Fisher's Exact test

The reviewers consider the statistical methods to be appropriate.

2. Indices

a. Reproductive indices - The following reproductive indices were calculated from breeding and parturition records of animals in the study:

Mating index = # inseminated females/# females co-housed with males x 100

Fertility index = # pregnant females/# inseminated females x 100

b. Offspring viability indices - The following viability (survival) indices were calculated from lactation records of litters in the study:

Live birth index = # live pups born per litter/total # pups per litter x 100

Viability index = # live pups on PND 4 pre-culling per litter/# live pups born per litter x 100

Lactation index = # live pups on PND 21 per litter/# live pups on PND 4 post-culling per litter x 100

3. Positive and historical control data – This study did not include concurrent positive controls, but references were made to previous studies to serve that purpose. It was stated that previous studies (MRID 42770301 [1993] and Bayer Report No. 110506 [2002]) with untreated animals and rats treated with substances that increase (triadimefon) and decrease (chlorpromazine) motor activity have established the sensitivity, reliability, and validity of the test procedures. Additional studies (MRID 45441302 [2001]) have been performed to establish test norms for the appropriate ages under these conditions and the effects of perinatal exposure to a reference chemical (methimazole) on activity in animals tested at these ages. Further studies (MRID 45441303 [2001]) were performed using reference substances (8OHDPAT, mCPP, and scopolamine) to validate the procedures and observers of the performing lab to conduct the FOB, auditory startle, passive avoidance, and water maze tests. Evidence of inter-observer reliability (MRID 42770301 [1993] and Bayer Report No. G200166 [2004]) is maintained for those individuals performing the observations.

The data from these referenced studies were not provided with the current study; however, summaries of the data from MRIDs 45464601, 45464602, 45441302, and 45441303, previously submitted to the Agency, were obtained and reviewed, and are included as Appendix I to this DER. It should be noted that the positive control data have been determined, by the Agency, to be marginal to inadequate.

II. RESULTS

A. PARENTAL ANIMALS

1. **Mortality and clinical and functional observations -** No treatment-related mortalities were observed during the study, all dams survived to scheduled sacrifice. No treatment-related clinical signs were observed during gestation or lactation. No adverse effects were observed in any FOB parameter at any time point.

2. **Body weight and food consumption -** Selected group mean body weights and body weight gains for pregnant and nursing dams are summarized in Table 2. During gestation, slight increases ($p \leq 0.05$) were noted in body weight (↑4-5%) and body weight gains (GD 0-20, ↑11%) at 12000 ppm. During lactation, body weights were increased ($p \leq 0.05$) by 4% at 120 and 1200 ppm on LD 0 and by 4-6% at 1200 and above on LD 21. Body weight gains (LD 0-21, calculated by reviewers) were also increased by 17-18% at 1200 and above. Food consumption in the treated groups was similar to controls during gestation and lactation. These differences in body weight and body weight gain were not considered to be treatment-related as the body weights of the treated animals were slightly higher than controls at the beginning of the study and minor increases in weight are not considered to be adverse.

TABLE 2. Selected mean (±SD) body weight and body weight gain in dams exposed to NNI-0001 in the diet from GD 6 through LD 21. [a]

Observations	Dose (ppm)			
	0	120	1200	12,000
Gestation (n=27-29)				
Body weight (g)				
GD 0	230.2±2.26	235.9±2.20	237.9±2.59	234.8±2.29
GD 13	268.3±2.37	276.7±2.96	279.5±2.99*(↑4)	278.2±2.64*(↑4)
GD 20	333.0±3.45	342.1±4.27	349.3±3.94**(↑5)	348.7±3.79**(↑5)
Body weight gain (g)				
GD 0-20	102.8±2.32	106.2±3.04	111.4±2.62*(↑8)	113.9±2.93*(↑11)
Lactation (n=20-29)				
Body weight (g)				
LD 0	259.7±2.38	268.9±2.84*(↑4)	270.7±2.99*(↑4)	266.3±2.75
LD 14	300.1±3.00	309.8±4.33	315.4±4.15	307.2±3.69
LD 21	290.4±3.30	301.3±3.64	306.6±3.43**(↑6)	302.5±3.63*(↑4)
Body weight gain (g)				
LD 0-21[b]	30.7	32.4	35.9 (↑17)	36.2 (↑18)

a Data were extracted from Tables 3 and 6 on pages 74 and 80 of the study report. Numbers presented parenthetically are percent difference from controls (calculated by reviewers).
b Calculated by reviewers from data within this table
* Statistically significantly different from controls at $p \leq 0.05$
** Statistically significantly different from controls at $p \leq 0.01$

3. **Test substance intake -** Mean daily mg test substance/kg body weight during the gestation and lactation periods are presented in Table 3. Based on these results the average intake during gestation and lactation was 0, 9.9, 99.5, and 979.6 mg/kg/day. Intake was based on maternal food consumption, body weight, and nominal dose.

TABLE 3. Test substance intake (mg/kg/day) in dams exposed to NNI-0001 in the diet from GD 6 through LD 21. [a]

Interval	Dose (ppm)			
	0	120	1200	12,000
Gestation (n=27-29)				
GD 6-13	0.0±0.00	10.4±0.22	102.1±1.91	1059.5±25.8
GD 13-20	0.0±0.00	10.7±0.26	102.9±1.49	1074.9±16.75
Lactation (n=19-23)				
LD 0-7	0.0±0.00	10.2±0.35	97.4±4.06	964.5±28.88
LD 7-14	0.0±0.00	9.2±0.17	97.9±1.73	882.0±19.37
LD 14-21	0.0±0.00	8.8±0.11	97.0±1.69	917.1±27.06

a Data were extracted from Table 8 on pages 84-85 of the study report.

4. **Reproductive performance** – Reproductive parameters were similar to controls in all dose groups (Table 4).

TABLE 4. Reproductive performance [a]

Observation	Dose (ppm)			
	0	120	1200	12,000
Number mated	30	30	30	30
Mating index (%)[b]	100.0	100.0	100.0	100.0
Fertility index (%)[b]	93.3	96.7	90.0	96.7
Gestation length (median # of days)	22.0	22.0	22.0	22.0

a Data were extracted from Table 1 on page 70 of the study report.
b **Mating index** = # inseminated females/# females co-housed with males x 100 and **fertility index** = # pregnant females/# inseminated females x 100

5. **Organ weight** - In the LD 21 dams, increases ($p \leq 0.05$) in absolute (↑26-34%) and relative (to body, ↑20-28%) liver weight were observed at 1200 ppm and above (Table 5). No treatment-related effect was noted in thyroid weight in the 12,000 ppm dams.

TABLE 5. Mean (±SD) liver weights in PND 21 dams [a]

Parameter	Dose (ppm)			
	0	120	1200	12,000
Absolute (g)	14.205±1.011	15.107±1.278	17.943±1.807* (↑26)	19.050±1.132* (↑34)
Relative (to body, %)	4.991±0.282	5.064±0.244	5.978±0.646* (↑20)	6.388±0.172* (↑28)

a Data were extracted from Text Table 19 on page 62 of the study report; n=10. Numbers presented parenthetically are percent difference from controls (calculated by reviewers).
* Statistically significantly different from controls at $p \leq 0.05$

B. OFFSPRING

1. **Viability and clinical signs -** Litter size and viability results from pups during lactation are summarized in Table 6. No compound related effects on any litter parameter were noted at any dose. Live birth, viability, and lactation indices were similar to controls at all doses. It was stated that the relatively high incidence of mortality at 12,000 ppm was due to the loss of

an entire litter and was not considered to be related to treatment.

TABLE 6. Litter size and viability [a]

Observation	Dose (ppm)			
	0	120	1200	12,000
No. of litters	20	22	21	23
Total number of pups born	230	255	245	274
Number born dead	1	0	0	2
Sex Ratio Day 0 (% %)	NR	NR	NR	NR
Mean litter size:				
PND 0	11	12	12	12
PND 4 [b]	11	12	12	12
PND 4 [c]	8	8	8	8
PND 21	8	8	8	8
# Deaths Days 4-24 [d]	4	1	3	7
Live birth index (%) [e]	99.7	100.0	100.0	99.3
Viability index (%) [e]	99.1	99.5	98.6	98.3
Lactation index (%) [e]	98.8	99.4	98.2	96.7

a Data were extracted from pages 43 and 45 and Table 9 on pages 75-76 of the study report.
b Pre-culling
c Post-culling
d Found dead, moribund, or missing
e **Live birth index** = # live pups born per litter/total # pups per litter x 100; **viability index** = # live pups on PND 4 pre-culling per litter/# live pups born per litter x 100; and **lactation index** = # live pups on PND 21 per litter/# live pups on PND 4 post-culling per litter x 100
NR Not reported

At 12,000 ppm, treatment-related ocular lesions were noted in pups of both sexes as follows. During pre-weaning (PND 0-21), the following effects on the eyes (# of litters affected/20-29 vs. 0 controls) were observed: (i) enlarged eyeball (1-9 litters on PND 15-21); (ii) corneal opacity (2-3 litters on PND 16-21); (iii) dark red (1-6 litters on PND 15-21); and (iv) exophthalmia (1 litter on PND 20-21, Table 6a). Throughout post-weaning (PND 22-72), the following effects on the eyes (# of animals affected/65-66 vs. 0 controls) were noted: (i) enlarged (9 males/12 females); (ii) general opacity (8 males/10 females); (iii) red (4 males/8 females; and (iv) exophthalmia (2 males, Table 6b). Additionally in the 1200 ppm males, one pup displayed enlarged eye, general opacity, and exophthalmia. No compound-related effects were noted at 1200 ppm in the females or at 120 ppm in either sex.

TABLE 6a. Incidence (# of liters affected) of ocular lesions in F_1 pups during pre-weaning (PND 0-21). [a]

Observation	Dose (ppm)			
	0	120	1200	12,000
Enlarged	0	0	0	9
Corneal opacity	0	0	0	3
Dark Red	0	0	0	6
Exophthalmia	0	0	0	1

a Data were extracted from Table 10 on page 90 of the study report; n=20-29.

TABLE 6b. Incidence (# affected) of ocular lesions in F_1 pups during post-weaning (PND 22-72). [a]								
Observation	Dose (ppm)							
	0	120	1200	12,000	0	120	1200	12,000
	Males				Females			
Enlarged [b]	0	0	1	9	0	0	0	12
General opacity [b]	0	0	1	8	0	0	0	10
Red [b]	0	0	0	4	0	0	0	8
Exophthalmia	0	0	1	2	1	0	0	0

a Data were extracted from Table 11 on pages 93-94 and Appendix XII on pages 310-376 of the study report; n=65-66.
b Observed either unilaterally in the left or right eye, or bilaterally in both eyes.

2. <u>Body weight</u> – Offspring pre-weaning body weights were decreased (p≤0.05) at 12,000 ppm (↓9% both sexes) at PND 21 (Table 7a). Body weight gains (Table 7b) were decreased (p≤0.05) during several pre-weaning intervals at 12,000 ppm (↓12-20%, males and ↓11-20%, females). Overall combined pup body weight gains (calculated by reviewers) were decreased by 11% at 12,000 ppm.

TABLE 7a. Mean (±SD) pre-weaning F_1 pup body weights (g) [a]				
Postnatal Day	Dose (ppm)			
	0	120	1200	12,000
Males				
0	5.9±0.10	6.0±0.09	6.2±0.10	6.3±0.10
4[b]	9.9±0.27	10.1±0.22	9.9±0.29	9.9±0.23
4[c]	9.9±0.27	10.0±0.23	9.9±0.29	9.9±0.25
17	38.4±0.68	39.1±0.75	38.0±0.73	36.1±1.02
21	49.7±0.88	50.0±0.91	47.9±0.79	45.1±1.25* (↓9)
Females				
0	5.6±0.10	5.7±0.09	5.8±0.10	5.9±0.10
4[b]	9.5±0.26	9.8±0.23	9.6±0.26	9.6±0.26
4[c]	9.6±0.26	9.8±0.23	9.5±0.26	9.6±0.26
17	37.5±0.62	38.4±0.63	37.0±0.70	35.3±1.11
21	48.4±0.86	49.1±0.85	46.8±0.77	44.0±1.39* (↓9)

a Data were extracted from Table 12 on pages 96-98 of the study report; n=20-23 litters. Numbers presented parenthetically are percent difference from controls (calculated by reviewers).
b Pre-culling
c Post-culling
* Statistically significantly different from controls at p≤0.05

TABLE 7b. Selected mean (±SD) pre-weaning F_1 pup body weight gains (g) [a]				
Interval (PND)	**Dose (ppm)**			
	0	**120**	**1200**	**12,000**
Males				
0-4	3.9±0.20	4.1±0.15	3.7±0.22	3.7±0.17
4-21	39.8±0.70	40.0±0.79	38.0±0.60	35.1±1.03**(↓12)
11-21	24.7±0.51	24.6±0.52	23.2±0.31*(↓6)	21.4±0.56**(↓13)
17-21	11.3±0.37	10.9±0.40	9.9±0.32*(↓12)	9.0±0.37**(↓20)
Females				
0-4	3.9±0.20	4.1±0.17	3.7±0.21	3.7±0.21
4-21	38.8±0.69	39.3±0.72	37.3±0.58	34.4±1.17**(↓11)
11-21	23.9±0.46	23.9±0.47	22.8±0.40	20.7±0.68**(↓13)
17-21	10.9±0.38	10.6±0.37	9.8±0.32	8.7±0.38**(↓20)
Combined				
Overall (0-21) gain [b]	43.1	43.5	41.2	38.3 (↓11)

a Data were extracted from Table 13 on pages 100-103 of the study report; n=20-23 litters. Numbers presented parenthetically are percent difference from controls (calculated by reviewers).
b Calculated by reviewers using combined data from Table 12 on pages 96-98 of the study report
* Statistically significantly different from controls at p≤0.05
** Statistically significantly different from controls at p≤0.01

Offspring post-weaning body weights remained decreased throughout the study at 12,000 ppm in both sexes; however, they only attained statistical significance (p≤0.05) in the males on Days 28 and 35 (↓7-8%; Table 8).

TABLE 8. Selected mean (±SD) post-weaning F_1 pup body weights (g) [a]				
Post-natal Day	**Dose (ppm)**			
	0	**120**	**1200**	**12,000**
Males				
28	79.5±6.8	81.8±7.9	77.6±7.6	72.8±10.3* (↓8)
35	127.5±8.6	130.3±12.0	124.9±10.7	118.1±14.9* (↓7)
42	173.3±10.2	176.7±14.3	171.3±13.4	163.0±18.7
70	325.0±14.9	330.2±21.6	327.5±23.1	310.4±33.3
Females				
29	78.6±7.8	80.3±6.3	76.1±6.6	74.0±7.1
50	158.9±9.6	158.8±9.9	156.5±11.7	155.2±8.8
71	200.1±12.3	200.3±11.2	199.0±16.3	198.3±11.1

a Data were extracted from Table 15 on pages 107-108 of the study report; n=20-23 litters. Numbers presented parenthetically are percent difference from controls (calculated by reviewers).
* Statistically significantly different from controls at p≤0.05

3. Developmental landmarks

a. Sexual maturation – The day of preputial separation was delayed greater than 2 days (p≤0.01) in the 1200 and 12,000 ppm males (47.5 and 48.7 treated vs. 44.9 controls). The day of vaginal patency was delayed (p≤0.01) greater than 2 days in the 12,000 ppm females (35.3 treated vs. 32.6 controls). Only 2 pups in the 12,000 ppm group did not display pupil constriction on PND 21.

TABLE 9. Sexual maturation (mean ±SD day of onset) in F_1 pups [a]

Parameter	Dose (ppm)			
	0	120	1200	12,000
Preputial separation	44.9±0.50	45.6±0.48	47.5±0.59**	48.7±0.73**
Vaginal patency	32.6±0.59	33.1±0.52	33.2±0.65	35.3±0.63**

a Data were extracted from Table 14 on page 105 of the study report; n=20-23 litters.
** Statistically significantly different from controls at $p \leq 0.01$

Reduced weight gain that resulted in decreased body weight during lactation persisted in high-dose males until study termination and in high-dose females for the first week measured after weaning. These differences from control were only statistically significant in males for the first two weeks measured after weaning. Lower body weights at the highest dietary level averaged 4-7% less than controls for males and 6% less than controls for females. Body weight was not affected by treatment in males or females at the two lower dietary levels. Therefore, it does not appear that body weight reduction is associated with the delay in sexual maturation.

b. Physical landmarks – Evaluation of physical landmarks (eye opening, pinna unfolding, incisor erupting) was not performed.

4. Behavioral assessments

a. Functional observational battery – Treatment-related FOB effects were limited to ocular lesions (corneal opacity, dark red eyes, and/or enlarged eyes) at 12,000 ppm (1-2 males and 1-4 females, Table 10). The lesions were observed beginning on PND 21 and persisted until PND 60 in both sexes. All other FOB findings were considered incidental and unrelated to treatment.

TABLE 10. Incidence (# affected [% incidence]) of ocular lesions in F_1 pups [a]

Observation	PND	Dose (ppm)							
		0	120	1200	12,000	0	120	1200	12,000
		Males (n=14-16)				Females (n=13-16)			
Corneal opacity	4	0	0	0	0	0	0	0	0
	11	0	0	0	0	0	0	0	0
	21	0	0	0	1 (6)	0	0	0	2 (13)
	35	0	0	0	2 (13)	0	0	0	2 (13)
	45	0	0	0	1 (7)	0	0	0	3* (21)
	60	0	0	0	0	0	0	0	3* (23)
Dark red	4	0	0	0	0	0	0	0	0
	11	0	0	0	0	0	0	0	0
	21	0	0	0	1 (6)	0	0	0	1 (7)
	35	0	0	0	1 (6)	0	0	0	2 (13)
	45	0	0	0	0	0	0	0	1 (7)
	60	0	0	0	0	0	0	0	0
Enlarged	4	0	0	0	0	0	0	0	0
	11	0	0	0	0	0	0	0	0
	21	0	0	0	2 (13)	0	0	0	2 (13)
	35	0	0	0	2 (13)	0	0	0	4* (27)
	45	0	0	0	1 (7)	0	0	0	3* (21)
	60	0	0	0	1 (7)	0	0	0	3* (23)

a Data were extracted from Table 17 on pages 135-194 of the study report.
* Statistically significantly different from controls at $p \leq 0.05$

b. Motor activity – No treatment-related differences in total session motor or locomotor activity were observed at any dose in either sex (Tables 11a and 11b). In the females, total session motor activity was increased ($p \leq 0.05$) by 92% at 12,000 ppm on PND 17 and by 28% at 120 ppm on PND 21. However, these findings were not considered to be related to treatment as they were not dose-dependent and/or transient. Interval motor and locomotor activity levels were similar to controls in both sexes at all time points. Levels of activity progressively increased with age compared to the levels on PND 13. Habituation was evident in both sexes at all ages.

TABLE 11a. Mean (±SD) total session motor activity (counts) in F_1 pups [a]

Interval (PND)	Dose (ppm)			
	0	120	1200	12,000
Males				
13	104±112	62±49	82±54	61±50
17	203±89	234±166	160±111	207±165
21	301±76	312±101	320±101	342±132
60	500±92	502±108	502±126	529±129
Females				
13	59±45	59±66	52±34	60±57
17	162±138	165±121	202±137	312±161* (↑92)
21	282±91	361±90* (↑28)	358±94	308±84
60	680±137	683±205	703±186	667±106

a Data were extracted from Text Table 12 on page 52 of the study report; n=15-16 pups.

TABLE 11b. Mean (±SD) total session locomotor activity (counts) in F_1 pups [a]

Interval (PND)	Dose (ppm)			
	0	120	1200	12,000
Males				
13	11±11	5±4	5±4	6±7
17	51±25	58±55	36±31	42±45
21	90±27	91±24	88±30	96±42
60	352±88	345±86	351±116	375±110
Females				
13	7±10	5±11	4±4	7±13
17	37±30	48±42	53±42	82±44
21	84±30	100±36	95±23	103±29
60	424±106	449±151	412±124	453±116

a Data were extracted from Text Table 13 on page 52 of the study report; n=15-16 pups.

c. Auditory startle reflex habituation – While for PND60 males there appeared to be a dose-response increase in acoustic startle peak amplitude in the blocks, when individual animal data was graphed onto a scatter plot (see appendix 1), the individual variability was clearly evident; based on results from the individual animal data visualized on the scatter plot, the increase in amplitude was judged not treatment-related but was largely driven by individual outliers. Therefore, no treatment-related effect on total session peak amplitude, latency, or habituation were observed in either sex at PND 22 or 60±2 (Table 12). The amplitude of the startle response increased with age in both sexes. At both ages,

habituation was apparent in the control animals as a decrease in amplitude during the course of the test session.

TABLE 12. Mean (±SD) overall (Blocks 1-5) acoustic startle peak amplitude (g) and latency to peak (msec) in F_1 rats [a]

Dose (ppm)	Parameter	Males		Females	
		PND 22	PND 60±2	PND 22	PND 60±2
0	Peak Amp.	38±17	162±87	26±8	108±63
	Latency	37±3	40±3	37±3	41±3
120	Peak Amp.	29±12	219±121	29±11	94±58
	Latency	38±6	40±3	38±3	38±4
1200	Peak Amp.	30±11	213±144	26±9	66±37
	Latency	37±2	39±3	38±4	40±3
12,000	Peak Amp.	33±11	228±145	35±18	95±56
	Latency	39±5	39±2	38±5	39±3

a Data were extracted from Text Table 14 on pages 54-55 of the study report; n=14-16.

d. <u>Learning and memory testing</u> – No compound-related effects were noted at any dose in either sex in the passive avoidance or M-maze tests (Tables 13 and 14). In the passive avoidance test, acquisition was evident in both sexes as a marked increase in the latency to cross for the second trial compared to the first trial. Retention was evident as a protracted delay to cross within the 180-sec time limit of the first trail compared to the first trial on the first test day, and a reduced number of trials-to-criterion on the second test day compared to the first day.

TABLE 13. Mean (±SD) passive avoidance performance in F_1 rats [a]

Session/Parameter		Dose (ppm)			
		0	120	1200	12,000
Males (n=14-16)					
Session 1 (PND 22) Learning	Trials to Criterion	3.1±1.1	3.1±0.3	3.0±0.0	3.3±0.8
	Latency Trial 1 (sec)	60.7±59.6	33.8±27.5	26.1±11.8	38.8±42.3
	Latency Trial 2 (sec)	180.0±0.0	174.8±20.7	180.0±0.0	176.7±9.8
	Failed to Meet Criterion	0	0	0	0
Session 2 (PND 29) Retention	Trials to Criterion	2.2±0.6	2.0±0.0	2.3±0.8	2.3±0.6
	Latency Trial 1 (sec)	179.2±2.9	180.0±0.0	169.4±28.9	158.7±47.4
	Latency Trial 2 (sec)	177.8±8.3	180.0±0.0	180.0±0.0	179.0±3.9
Females (n=15-16)					
Session 1 (PND 22) Learning	Trials to Criterion	3.1±0.3	3.0±0.4	3.0±0.0	3.2±0.5
	Latency Trial 1 (sec)	53.8±48.2	43.6±55.2	30.2±21.4	28.6±23.2
	Latency Trial 2 (sec)	176.8±12.7	174.4±22.3	180.0±0.0	177.4±10.2
	Failed to Meet Criterion	0	0	0	0
Session 2 (PND 29) Retention	Trials to Criterion	2.0±0.0	2.5±0.8	2.4±0.8	2.3±0.6
	Latency Trial 1 (sec)	180.0±0.0	174.7±20.3	175.0±20.2	163.7±38.5
	Latency Trial 2 (sec)	180.0±0.0	168.0±30.4	163.0±39.5	178.0±8.1

a Data were extracted from Text Table 16 on page 57 of the study report.

In the water maze test, acquisition was evident in both sexes as a progressive decrease in the average time to escape over successive trials. Retention was evident as a reduction in the number of trials-to-criterion and a shorter trial duration for the first trial compared to the first trial of acquisition. The increased ($p \leq 0.05$) second trial duration during acquisition noted in the 12,000 ppm males was considered incidental and unrelated to treatment as the difference was small and within the range of historical controls (11.3 to 21.4 seconds).

TABLE 14. Mean (±SD) water maze performance in F_1 rats [a]

Session/Parameter		Dose (ppm)			
		0	120	1200	12,000
Males					
Session 1 (PND 60±2) Learning	Trials to Criterion	5.9±1.2	6.4±1.8	6.9±2.5	7.8±3.4
	Trial 1 Errors	0.6±0.9	0.7±0.9	0.4±0.8	0.8±1.1
	Latency Trial 1 (sec)	15.7±16.1	14.9±9.8	19.7±14.4	18.1±14.2
	Trial 2 Errors	0.2±0.4	0.4±0.6	0.6±0.9	0.8±1.1
	Latency Trial 2 (sec)	9.7±5.4	9.8±5.4	11.4±5.5	17.3±13.1*
	Failed to Meet Criterion	0	0	0	1
Session 2 (PND 67±2) Retention	Trials to Criterion	5.5±0.8	5.6±1.4	6.3±2.1	6.0±1.7
	Trial 1 Errors	0.6±1.0	0.1±0.5	0.6±1.0	0.9±1.5
	Latency Trial 1 (sec)	12.3±11.3	6.8±4.4	10.6±14.1	11.4±13.7
	Trial 2 Errors	0.0±0.0	0.0±0.0	0.3±0.7	0.1±0.5
	Latency Trial 2 (sec)	4.0±1.7	3.8±1.2	5.3±4.3	4.0±2.0
Females					
Session 1 (PND 60±2) Learning	Trials to Criterion	7.4±3.1	7.0±2.2	8.8±3.1	7.9±2.2
	Trial 1 Errors	1.1±1.3	0.9±1.2	1.1±1.1	1.5±1.1
	Latency Trial 1 (sec)	19.6±14.5	18.0±14.3	17.8±11.2	24.9±15.4
	Trial 2 Errors	0.7±1.6	0.6±0.9	1.0±1.0	0.8±0.7
	Latency Trial 2 (sec)	12.9±10.9	13.0±9.1	15.6±13.9	14.4±9.1
	Failed to Meet Criterion	1	0	1	0
Session 2 (PND 67±2) Retention	Trials to Criterion	7.3±3.3	7.0±2.5	6.5±1.8	7.7±3.6
	Trial 1 Errors	0.3±0.7	0.2±0.5	0.5±0.8	0.5±0.7
	Latency Trial 1 (sec)	8.1±8.7	9.1±10.5	7.8±5.1	9.7±6.1
	Trial 2 Errors	0.4±0.9	0.5±1.2	0.2±0.6	0.2±0.4
	Latency Trial 2 (sec)	7.5±9.6	9.2±11.6	6.4±5.8	6.2±3.6

a Data were extracted from Text Table 17 on pages 58-59 of the study report; n=14-16.

5. <u>Ophthalmology</u> – Although corneal opacities were noted on PND 50 (M: 2, 1, 0, 3; F: 1, 1, 1, 1; in the control, 120, 1200, and 12,000 ppm groups, respectively), it was stated that no compound-related ocular lesions were noted in either sex. These results differ from the results for clinical observations, FOB, and gross pathology, where compound-related corneal opacities and exophthalmos were evident in the 12,000 ppm pups. It was stated that these differences, along with other ocular lesions, were not attributed to treatment, because of the low incidence (1 female and 3 males account for all the findings at 12,000 ppm).

6. Postmortem results

a. Organ weights – No treatment-related effects were noted in terminal body weights or absolute and relative (to body) brain weights on PNDs 21 or 75±5 in either sex (Table 15).

TABLE 15. Mean (±SD) brain weight data from perfused and non-perfused F_1 rats [a]

Parameter	Dose (ppm)			
	0	120	1200	12,000
Males				
PND 21 (Perfused)				
Terminal body weight (g)	48.5±5.7	47.6±3.2	50.1±4.0	46.9±3.3
Brain weight (g)	1.428±0.057	1.378±0.083	1.483±0.086	1.394±0.049
Brain-to-body weight ratio (%)	2.985±0.388	2.903±0.168	2.974±0.295	2.987±0.257
PND 75±5 (Termination – Perfused)				
Terminal body weight (g)	362.4±28.9	349.0±29.7	351.4±32.1	333.9±33.8
Brain weight (g)	1.918±0.091	1.856±0.073	1.933±0.110	1.842±0.089
Brain-to-body weight ratio (%)	0.531±0.039	0.535±0.045	0.553±0.041	0.556±0.049
PND 75±5 (Termination – Non-perfused)				
Terminal body weight (g)	339.0±20.1	342.2±42.5	354.4±28.6	334.9±22.7
Brain weight (g)	2.022±0.058	1.973±0.089	2.006±0.054	1.980±0.099
Brain-to-body weight ratio (%)	0.598±0.029	0.585±0.079	0.569±0.045	0.594±0.051
Females				
PND 21 (Perfused)				
Terminal body weight (g)	48.4±3.7	51.6±4.4	46.7±3.6	44.6±5.3
Brain weight (g)	1.364±0.085	1.408±0.036	1.386±0.092	1.363±0.066
Brain-to-body weight ratio (%)	2.829±0.207	2.747±0.243	2.977±0.213	3.084±0.280
PND 75±5 (Termination – Perfused)				
Terminal body weight (g)	206.8±19.1	209.7±15.9	206.1±17.4	208.2±14.6
Brain weight (g)	1.757±0.074	1.822±0.078	1.754±0.086	1.795±0.041
Brain-to-body weight ratio (%)	0.857±0.099	0.872±0.055	0.857±0.087	0.866±0.060
PND 75±5 (Termination – Non-perfused)				
Terminal body weight (g)	206.3±11.6	211.5±15.3	209.1±22.7	207.4±12.1
Brain weight (g)	1.773±0.143	1.827±0.133	1.831±0.131	1.886±0.079
Brain-to-body weight ratio (%)	0.861±0.069	0.866±0.068	0.882±0.086	0.911±0.050

a Data were extracted from Text Table 18 on page 61 of the study report; n=10.

b) Neuropathology

1. **Macroscopic examination** – No gross lesions or significant differences in cerebellum and cerebrum lengths were observed on PND 21 or at termination in either sex.

2. **Microscopic examination** – At 12,000 ppm, the following compound-related microscopic effects (# affected/10 vs. 0 controls, unless otherwise stated) were noted in the eye and optic nerve (Table 16): (i) retinal degeneration (2 males and 1 female); (ii) hemorrhage (3 males vs. 1 control); (iii) cataract (2 males and 1 female); and (iv) atrophy of the optic nerve (3 males and 1 female). No other treatment-related microscopic lesions were noted at any dose in either sex.

TABLE 16. Incidence of microscopic lesions (# affected/10) in F_1 rats [a]

Observation		Dose (ppm)			
		0	12,000	0	12,000
		Males		Females	
Eye	Retinal degeneration	0	2	0	1
	Hemorrhage	1	3	0	0
	Cataract	0	2	0	1
Optic nerve, Atrophy		0	3	0	1

a Data extracted from Table MP2-SUM on pages 884-887.

No treatment-related differences in morphometric brain measurements were noted in the 12,000 ppm animals compared to controls on PND 21 or 75±5 (Tables 17a and 17b).

TABLE 17a. Mean (±SD) morphometric brain measurements (mm) in male F_1 rats [a]

Parameter	Dose (ppm)			
	0	120	1200	12,000
PND 21				
Cerebrum Length	13.56±0.27	13.29±0.36	13.86±0.37	13.51±0.24
Cerebellum Length	7.37±0.31	7.30±0.40	7.36±0.39	7.22±0.40
Frontal Cortex	1.72±0.01	NM	NM	1.75±0.01
Parietal Cortex	1.90±0.00	NM	NM	1.89±0.00
Caudate Putamen	3.13±0.02	NM	NM	3.01±0.02
Hippocampal Gyrus	1.62±0.00	NM	NM	1.69±0.01
Cerebellum Height	4.12±0.06	NM	NM	4.15±0.04
PND 75±5 (Termination)				
Cerebrum Length	14.76±0.37	14.62±0.33	14.60±0.32	14.48±0.40
Cerebellum Length	7.79±0.39	7.73±0.41	7.77±0.23	7.74±0.18
Frontal Cortex	1.71±0.02	NM	NM	1.72±0.01
Parietal Cortex	1.86±0.01	NM	NM	1.88±0.00
Caudate Putamen	3.46±0.01	NM	NM	3.37±0.04
Hippocampal Gyrus	1.77±0.01	NM	NM	1.84±0.01
Cerebellum Height	4.56±0.10	NM	NM	4.59±0.22

a Data were extracted from Text Table 20 on pages 63-64; n=9-10.
NM Not measured

TABLE 17b. Mean (±SD) morphometric brain measurements (mm) in female F_1 rats [a]				
Parameter	Dose (ppm)			
	0	120	1200	12,000
PND 21				
Cerebrum Length	13.40±0.36	13.54±0.32	13.54±0.29	13.23±0.29
Cerebellum Length	7.26±0.36	7.10±0.52	7.44±0.39	7.12±0.43
Frontal Cortex	1.73±0.00	NM	NM	1.73±0.01
Parietal Cortex	1.86±0.01	NM	NM	1.87±0.01
Caudate Putamen	2.95±0.02	NM	NM	3.02±0.02
Hippocampal Gyrus	1.66±0.02	NM	NM	1.69±0.02
Cerebellum Height	4.08±0.02	NM	NM	4.20±0.07
PND 75±5 (Termination)				
Cerebrum Length	14.18±0.34	14.26±0.40	14.17±0.21	14.25±0.41
Cerebellum Length	7.86±0.46	7.89±0.40	7.69±0.29	7.71±0.38
Frontal Cortex	1.68±0.01	NM	NM	1.68±0.01
Parietal Cortex	1.78±0.00	NM	NM	1.80±0.00
Caudate Putamen	3.37±0.01	NM	NM	3.29±0.04
Hippocampal Gyrus	1.67±0.02	NM	NM	1.68±0.04
Cerebellum Height	4.49±0.17	NM	NM	4.55±0.04

a Data were extracted from Text Table 20 on pages 63-64; n=10.
NM Not measured

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS CONCLUSIONS –

The investigators concluded that dietary administration of NNI-0001 from GD 6 through LD 21 induced the following effects at 1200 ppm and above: (i) increased absolute and relative (to body) liver weight in the dams; (ii) ocular lesions (enlarged eyeball, exophthalamus, ocular opacities) in the pups; (iii) decreased pre-weaning body weight gain in both sexes; and (iv) delayed balanopreputial separation. Additional effects noted at 12,000 ppm included decreased post-weaning body weight and body weight gain in both sexes, and delayed vaginal patency. No evidence of developmental neurotoxicity was observed at any dose in either sex.

B. REVIEWER COMMENTS –

Mean daily mg test substance/kg body weight during the gestation and lactation periods Averaged 0, 9.9, 99.5, and 979.6 mg/kg/day. based on maternal food consumption, body weight, and nominal dose.

Maternal Effects:
No treatment-related mortalities were observed during the study, all dams survived to scheduled sacrifice. No treatment-related clinical signs were observed during gestation or lactation. No adverse effects were observed in any FOB parameter at any time point.

During gestation, slight increases ($p \leq 0.05$) were noted in body weight (↑4-5%) and body weight gains (GD 0-20, ↑11%) at 12000 ppm. During lactation, body weights were increased ($p \leq 0.05$) by 4% at 120 and 1200 ppm on LD 0 and by 4-6% at 1200 and

above on LD 21. Body weight gains (LD 0-21, calculated by reviewers) were also increased by 17-18% at 1200 and above. Food consumption in the treated groups was similar to controls during gestation and lactation. These differences in body weight and body weight gain were not considered to be treatment-related as the body weights of the treated animals were slightly higher than controls at the beginning of the study and minor increases in weight are not considered to be adverse. In the LD21 dams, increases ($p \leq 0.05$) in absolute (↑26-34%) and relative (to body, ↑20-28%) liver weight were observed at 1200 ppm and above.

Reproductive parameters were similar to controls in all dose groups.

Pup effects.:
Offspring pre-weaning body weights were decreased ($p \leq 0.05$) at 12,000 ppm (↓9% both sexes) at PND 21. Body weight gains were decreased ($p \leq 0.05$) during several pre-weaning intervals at 12,000 ppm (↓12-20%, males and ↓11-20%, females). Overall combined pup body weight gains (calculated by reviewers) were decreased by 11% at 12,000 ppm.

Sexual maturation – The day of preputial separation was delayed ($p \leq 0.01$) in the 1200 and 12,000 ppm males (47.5 and 48.7 treated vs. 44.9 controls) greater than 2 days. The day of vaginal patency was delayed ($p \leq 0.01$) in the 12,000 ppm females (35.3 treated vs. 32.6 controls) greater than 2 days. Only 2 pups in the 12,000 ppm group did not display pupil constriction on PND 21.

Functional observational battery – Treatment-related FOB effects were limited to ocular lesions (corneal opacity, dark red eyes, and/or enlarged eyes) at 12,000 ppm (1-2 males and 1-4 females). The lesions were observed beginning on PND 21 and persisted until PND 60 in both sexes. All other FOB findings were considered incidental and unrelated to treatment.

At 12,000 ppm, treatment-related ocular lesions were noted in pups of both sexes as follows. During pre-weaning (PND 0-21), the following effects on the eyes (# of litters affected/20-29 vs. 0 controls) were observed: (i) enlarged eyeball (1-9 litters on PND 15-21); (ii) corneal opacity (2-3 litters on PND 16-21); (iii) dark red (1-6 litters on PND 15-21); and (iv) exophthalmia (1 litter on PND 20-21). Throughout post-weaning (PND 22-72), the following effects on the eyes (# of animals affected/65-66 vs. 0 controls) were noted: (i) enlarged (9 males/12 females); (ii) general opacity (8 males/10 females); (iii) red (4 males/8 females; and (iv) exophthalmia (2 males). Additionally in the 1200 ppm males, one pup displayed enlarged eye, general opacity, and exophthalmia. No compound-related effects were noted at 1200 ppm in the females or at 120 ppm in either sex.

Microscopic examination – At 12,000 ppm, the following compound-related microscopic effects (# affected/10 vs. 0 controls, unless otherwise stated) were noted in the eye and optic nerve: (i) retinal degeneration (2 males and 1 female); (ii) hemorrhage (3 males vs. 1 control); (iii) cataract (2 males and 1 female); and (iv) atrophy of the optic nerve (3 males and 1 female). No other treatment-related microscopic lesions were noted at any dose in either sex.

Maternal LOAEL = 99.5 mg/kg/day based on increased liver weights. Increased liver

weight in isolation is not considered an "adverse" effect, but considering the consistent observation of liver toxicity (e.g., centrilobular hepatocyte fatty change, hypertrophy, increase in liver enzymes, foci of cellular alterations) demonstrated across multiple durations and species at similar doses, the weight-of-evidence supports this effect as an "adverse" finding and thus, a firm basis for the LOAEL. Maternal NOAEL= 9.9 mg/kg/day.

Offspring LOAEL = 99.5 mg/kg/day based on delayed balanpreputial separation. The Offspring NOAEL = 9.9 mg/kg/day.

This study is classified (**acceptable/non-guideline**) and satisfies the guideline requirement; OPPTS 870.6300, '83-6, OECD 426 (draft) for a developmental neurotoxicity study in rats.

C. <u>STUDY DEFICIENCIES</u> - None

APPENDIX I

BAYER CORP

MAIN FORM

Laboratory	Bayer Corp., Stilwell, KS			
Study No.	**MRID**	**TRX**	**Year**	**Citation**
1	45441302		2001	1. Sheets,L.P. and Lake,S.G. (2001) Method Validation Study for a Developmental Neurotoxicity Screen: Untreated (Nomative) and Perinatal Methimazole Treatment in Wistar Rats. Bayer Corporation, Stilwell, KS, Laboratory Study Number 98-982-RR, Feb 9, 2001. 973 p. MRID 45441302.
2	45441303		2001	2. Sheets,L.P. (2001) Historical Control and Method Validation Studies in rats for a Developmental Neurotoxicity Screening Battery (Auditory Startle Habituation and Cognitive Function (Passive Avoidance and Water Maze Conditioning). Bayer Corporation, Stilwell, KS, Laboratory Study Number 98-992-VV, 98-992-UM, 98-992-WC, 99-D82-AF, Feb 9, 2001. 191 p. MRID 45441303.
3	45464601		1999	3. Sheets,L.P. and Gilmore,R.G. (1999) Verification of Personnel Training to Perform a Functional Observational Battery with Rats. Unpublished study prepared by Bayer Corporation, Stilwell, KS. Laboratory Study Number 97-962-LG. September 16, 1999. 94 p. MRID 4544601
4	45464602		2000	4. Sheets,L.P. and Armintrout,G.L. (2000) A Motor Activity Historical Control and Method Validation Study using Triadimefon and Chlorpromazine in Wistar rats. Unpublished study prepared by Bayer Corporation, Stilwell, KS. Laboratory Study Number 97-482-OU. June 19, 2000. 56 p. MRID 45464602

Positive Control Review Form

Testing Laboratory	Bayer Co., Stilwell, KS	1. Sheets,L.P. and Lake,S.G. (2001) Method Validation Study for a Developmental Neurotoxicity Screen: Untreated (Nomative) and Perinatal Methimazole Treatment in Wistar Rats. Bayer Corporation, Stilwell, KS, Laboratory Study Number 98-982-RR, Feb 9, 2001. 973 p. MRID 45441302.	
Positive Control	Methimazole (d)		
Date of Positive Control Data	2001		
Species/Strain	Wistar rats		
QA Review (yes/no)	Yes	Date of Review	November 2002

Methods	Method Codes	Data Present?	Age Relevant?	Age (days)	Dose Levels	Sexes (m/f)	Group Size	Effects (y/n)	Comments
Dev Landmarks (PDX X)	PS,VO,EO,SR,PI, ASR	raw, means	yes	var	1	m,f	20	y	
FOB		yes	yes	4.1e+10	1	m,f	15	n	
Motor Activity	PH (Columbus)	yes	yes	13-17, 60	1	m,f	20	y	
Startle	CO	raw, means	yes	223860	1	m,f	20	y	
Learn/Memory	PA MZ	raw, means	yes	24-31 60-67	1	m,f	20	n y	
Std Histopath		yes	yes	11	1	m,f	10	n	
Morphometrics		raw, means	yes	11, 70	1	m,f	10	y, y	
Thyroid Hormone and Histopath		yes	yes	1170	1	m,f	10	y hormone y histopath	

Is data report adequate (individual data, methods, etc)?	Good example of data reporting. Separate submission in standard format. QA, summaries and raw data
Methods/Results	**Methods:** One dose of methimazole from GD16 to PND10 at 0.1 mg/ml in the drinking water. Standard methods used in the Bayer lab for DNT studies. **Results:** 14% decrease in maternal body weight and decrease in pup weights postnatally recovery in males not in females by PND~60. Most endpoints were affected. Exceptions included: no change in FOB, no change in PND24 passive avoidance, and no evidence of histopathology from standard subjective assessments. Motor activity was only affected on PND 13 and no other ages. No effect on startle on PND23, increases on PND 38 and 60 only in males. Only effect in males on learning phase in water maze test. No effects in females. There was an 84% decrease in T4 and a 16% decrease in T3.
Summary	**Summary**: It appears to be adequate data to support proficiency for developmental exposure to one agent. Problems include: only decrease seen in MA testing and only at one age, no increases; no effects on FOB measures, PND24 learning/memory testing or std histopath. Only effects in one sex in learning portion of water maze, no effects on retention testing. Note that other data from this lab support proficiency with adult motor activity. **Overall Conclusion**: Proficiency = marginal.

Positive Control Review Form

Testing Laboratory	Bayer Co., Stilwell, KS	2. Sheets,L.P. (2001) Historical Control and Method Validation Studies in rats for a Developmental Neurotoxicity Screening Battery (Auditory Startle Habituation and Cognitive Function (Passive Avoidance and Water Maze Conditioning). Bayer Corporation, Stilwell, KS, Laboratory Study Number 98-992-VV, 98-992-UM, 98-992-WC, 99-D82-AF, Feb 9, 2001. 191 p. MRID 45441303.	
Positive Control	1. 8-hydroxy-(di-n-propylamino) piperazine HBr (8OH-DPAT) 2. 1-m-chlorophenyl piperazine HCl (mCPP) 3. Scopolamine HBr		
Date of Positive Control Data	2001		
Species/Strain	Male and female Crl:Wistar(HAN,BR) rats		
QA Review (yes/no)	Yes	Date of Review	November 2002

Methods	Method Codes	Data Present?	Age Relevant?	Age (days)	Dose Levels	Sexes (m/f)	Group Size	Effects (y/n)	Comments
Dev Landmarks (PND X)									
FOB									
Startle	CO	yes	no	30	5 mg/kg	m	8	n	mCPP
Startle	CO	yes	no	32	0.5, 1.0 mg/kg	f	10	y	8OH-DPAT
Startle	CO	yes	no	30	0.25 mg/kg	m	8	n	8OH-DPAT
Learn/Memory	PA MZ	yes yes	n y	3556		m m,f	1212	yes no	scopolamine, MZ=water m-maze
Std Histo									
Morphometrics									

Is data report adequate (individual data, methods, etc)?	Data presentation is adequate.
Methods/Results	**Methods:** For startle used a fairly standard 50 trial habituation paridigm. Recorded peak amplitude. Testing was immediately post-dosing. Cognitive testing used an M-maze and one dose of scopolamine, 1.0 mg/kg administered between 30 and 60 minutes prior to testing. Training consisted of 15 trials. Retention testing was conducted 24 hours later. Variables were trials to criterion, number of errors and latency to goal. Passive avoidance used a standard paridigm: trials to criterion was maxed out at 15. **Results:** For startle there was no effect of the mCPP 8-OHDPAT caused an increase at the highest dose. Results for the water maze testing are not very good. There were no statistically significant effects on any measure in males, in females there was a decrease in latency (??), and an increase in the number that failed to meet criterion (controls = 2; scoploamine = 5). For passive avoidance the data are not very impressive. For the learning phase there was a small increase in the trials to criterion and a small decrease in the latency on trial 2, but not trial 1. For retention testing there was a small increase in trials to criterion, a decrease in latency on trial 1 and no effect on trial 2. Not very big effects compared to published literature on scopolamine and passive avoidance.
Summary	**Summary:** These data alone are marginal to non-acceptable as evidence of proficiency. There are only males for the PA testing and the effects of 1.0 mg/kg scopolamine are rather small. M-maze performance was not affected at all in males and affected only slightly in females. Startle shown to be increased, but not decreased by reference compounds used previously by the author. **Overall Conclusion**: Proficiency = not demonstrated for startle, PA, or M-maze.

Positive Control Review Form

Testing Laboratory	Bayer Co., Stilwell, KS	3. Sheets,L.P. and Gilmore,R.G. (1999) Verification of Personnel Training to Perform a Functional Observational Battery with Rats. Unpublished study prepared by Bayer Corporation, Stilwell, KS. Laboratory Study Number 97-962-LG. September 16, 1999. 94 p. MRID 45464601	
Positive Control	Carbaryl		
Date of Positive Control Data	1999		
Species/Strain	Wistar rats		
QA Review (yes/no)	Yes	Date of Review	November 2002

Methods	Method Codes	Data Present?	Age Relevant?	Age (days)	Dose Levels	Sexes (m/f)	Group Size	Effects (y/n)	Comments
Dev Landmarks (PND X)									
FOB		yes	yes/no	63	2 (15,30 mg/kg)	m	6	yes	carbaryl
Motor Activity									
Startle									
Learn/Memory									
Std Histopath									
Morphometrics									

Is data report adequate (individual data, methods, etc)?	Good example of data reporting. Separate submission in standard format. QA, summaries and raw data
Methods/Results	**Methods:** Standard FOB with ranking scales plus grip strength and footsplay. Carbaryl administered ip, 15-70 min prior to testing. Five technicians rated each animal and interobserver reliability was assessed. **Results:** Dose response was apparent. A variety of endpoints were affected as would be expected with carbaryl. Some endpoints were not affected. Good overall agreement between observers.
Summary	**Summary:** Data for FOB are inadequate: only data for males, small group size (n=6), one dose, only adults. Only one compound and not all endpoints affected. Same data as was submitted for adult neurotox studies. **Overall Conclusion:** unacceptable

Positive Control Review Form

Testing Laboratory	Bayer Co., Stilwell, KS	4. Sheets,L.P. and Armintrout,G.L. (2000) A Motor Activity Historical Control and Method Validation Study using Triadimefon and Chlorpromazine in Wistar rats.. Unpublished study prepared by Bayer Corporation, Stilwell, KS. Laboratory Study Number 97-482-OU. June 19, 2000. 56 p. MRID 45464602	
Positive Control	triadimefon, chlorpromazine		
Date of Positive Control Data	2000		
Species/Strain	Wistar rats		
QA Review (yes/no)	Yes	Date of Review	November 2002

Methods	Method Codes	Data Present?	Age Relevant?	Age (days)	Dose Levels	Sexes (m/f)	Group Size	Effects (y/n)	Comments
Dev Landmarks (PND X)									
FOB									
Motor Activity	F8	yes	yes/no	70	0, 200 mg/kg	m	12	yes	triadimefon
	F8	yes	yes/no	70	0, 2 mg/kg	m	12	yes	chlorpromazine
Learn/Memory									
Std Histopath									
Morphometrics									

Is data report adequate (individual data, methods, etc)?	Good example of data reporting. Separate submission in standard format. QA, summaries and raw data.
Methods/Results	**Methods:** Triadimefon (90 min prior to testing, po) and chlorpromazine (60 min prior, ip) were administered to 70 day old Wistar rats. Testing in figure-8 mazes lasted 90 min, summed in 10 min bins. Data analyzed by SAS. **Results:** Triadimefon resulted in increased activity of about 300% and a decrease in habituation. Chlorpromazine resulted in decreased activity, about 50% for total counts.
Summary	**Summary:** Triadimefon and chlorpromazine data are inadequate due to males only and only adults at 70 days of age. Data demonstrate ability to detect decreases and increases, as well as decreased habituation. Sensitivity is unknown due to lack of dose response. **Overall Conclusion:** Marginal.